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RESEARCH ARTICLE

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COMPARATIVE STUDY ON CONCRETE HEALING ACTIVITY OF *BACILLUS* SP.

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ABSTRACT

Concrete is a composite material widely used in construction industry. But the most common problem associated with concrete structures is cracking. A biological method of crack healing also known as Bio cementation was investigated in the present study using *Sporosarcina globiospora* and *Bacillus subtilis*. The bacteria hydrolyzes urea in the microenvironment producing ammonia and calcium carbonate, the latter being responsible for filling up cracks and deformations in the concrete structure. 70 mL concentration of bacterial samples significantly improved the compressive strength of concrete. The results also prove that increasing the curing period of concrete cubes have a positive impact on their strength. The addition of ConCure (curing compound) to the cement mix improved mechanical strength of the concrete. The slight decrease in pH of cured water from bacterial samples confirmed carbonic ion production due to urease activity. Ammoniac nitrogen production was greater for *B. subtilis* as compared to *S.globiospora*. Flame and ammonium oxalate tests confirmed calcium carbonate production. *B. subtilis* produced better results for compressive testing and urease activity as compared to *S.globiospora.*

Keywords- Bio cementation, *Bacillus subtilis*, curing, *Sporosarcina globiospora*, Urease.

INTRODUCTION

Concrete is a composite material consisting of cement (which acts as a binder for coarse or fine aggregates), water and some other components for improving the mechanical strength. The simplest representation of concrete is given by, Concrete as a combination of filler and binder. According to the type of binder used, there are three types of concrete used which include Portland cement, asphalt and epoxy concrete. For Portland cement, (Cement+ Admixture +Water) → cement paste+ fine aggregate→ Mortar+ coarse aggregate→ Concrete. Admixtures are additional materials (examples-super plasticizers/ConCure) added to other concrete components which gives specific characteristics to the concrete not available for normal concrete mixes [5].

As the liquid content in the concrete evaporates, the concrete structure is vulnerable to cracking. Bio cementation also known as microbiologically induced calcite precipitation (MICP) provides a feasible cost-effective solution to this problem.. Bacteria induce precipitation of calcium carbonate by the production of a urease enzyme. This enzyme uses urea as the sole nitrogen source, catalyzing the hydrolysis of urea into ammonium

which increases the pH. This results in calcium carbonate precipitation [19].

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow^{urease} 2NH_4^+ + CaCO_3$$

Bacillus subtilis is a gram-positive, rod-shaped, catalase-positive, endospore producing facultative anaerobic bacterium found in the soil and gastrointestinal tract of ruminants and humans. The optimum conditions for their growth are temperature in the range of 25-45 °C, pH of 7-11 in the presence of 7% sodium chloride [4, 6, 13 and 14].

Sporosarcina globisporus, formerly known as *Bacillus globisporus*, is a gram-positive, aerobic, round spore-forming *Bacillus*. Strains of this species were originally described in 1967 and were found to be fairly similar to the species *Bacillus pantothenticus*. The species was later reassigned to the genus *Sporosarcina* along with the species *Bacillus psychrophilus* and *Bacillus pasteurii*. The bacterium is a rod-shaped, motile microorganism has white colored colonies with terminal spores. The optimum growth conditions include pH of 10 and temperature in the range between 20-25 ° C. *Sporosarcina globiospora* grows an aerobically and produces oxidase and urease but cannot hydrolyze casein and starch [3].

Considerable research has taken place in the field of bacterial concrete [18]. Some of the bacteria investigated for urease activity were *Bacillus sphaericus* [15], *Sporosarcina pasteurii* and *Pseudomonas aeruginosa* [1, 2, 16 and 17].

The present study is aimed at comparing the ureolytic properties of a novel bacterium *Sporosarcina globiospora* an established urease producing bacterium *Bacillus subtilis*.

Materials and Methods

Preparation of bacterial culture

Sterile Petri plate cultures of *Bacillus subtilis* were obtained from the department of Biotechnology, MIT Manipal. 12.5 g of nutrient broth (media) was mixed in 500 mL of distilled water in a conical flask. The flask was made airtight by cotton plug and autoclaved at 121 °C, 15 psi for 15 minutes. The flask was kept in the laminar chamber (for maintaining sterile conditions) for cooling. Then a loopful of *B. subtilis* culture from the plate was inoculated in the nutrient broth and the flask was incubated in a rotary shaker at 150-200 rpm overnight. The appearance of turbidity overnight confirms bacterial growth.

Lyophilized culture of *Sporosarcina globiospora* was obtained from Microbial type culture collection (MTCC, Chandigarh, India). 12.5 g of nutrient broth (media) was mixed in 500 mL of distilled water in a conical flask. The flask was made airtight by cotton plug and autoclaved at 121 °C, 15 psi for 15 minutes. The flask was kept in the laminar chamber (for maintaining sterile conditions) for cooling. Then a small amount of the bacterium was inoculated in nutrient broth and the flask was incubated in a rotary shaker at 150-200 rpm overnight. The appearance of turbidity overnight confirms bacterial growth.10^5 cells/mL bacterial concentration was cast with the cement mix.

Preparation of concrete molds

The concrete cubes were prepared as per specific Indian standards (IS). Ordinary Portland cement (O.P.C) (43 grade) and M30 cement conforming to [9] standards is used with normal aggregate size of 20 mm. The maximum water-cement content is 0.50, minimum cement content is 250 kg/m^3 and crushed aggregates are used. Specific gravity of the cement is 3.16 and that of coarse, fine aggregates are 2.71 and 2.67 respectively. Water absorption of coarse and fine aggregates is 0.62 and 1.34 respectively [9]. The target mean strength is taken as 38.25 N/mm^2. The maximum water-cement ratio is taken as 0.455. The specific gravity of bacterial culture is 1.01[10].

1) Control sample

Table 1- Mix design for control sample

Cement	430.00 kg/m^3
Water	195.65 kg/m^3
Fine Aggregate	620.00 kg/m^3
Coarse Aggregate	1130.00 kg/m^3
Water-Cement ratio	0.455

2) 30 mL bacterial sample

Liquid culture used in mix (kg/m^3):

$$\frac{29.98}{1.01} = 29.68$$

Table 2- Mix design for 30 mL bacterial sample

Cement	430.00 kg/m^3
Water	165.67 kg/m^3
Fine Aggregate	620.00 kg/m^3

Coarse Aggregate	1130.00 kg/m^3
Water-Cement ratio	0.455
Sporosarcina globiospora (liquid culture)	29.68 kg/m^3

3) 50 mL bacterial sample

Liquid culture used in mix (kg/m^3):

$\frac{50.49}{1.01} = 49.99$

Table 3- Mix design for 50 mL bacterial sample

Cement	430.00 kg/m^3
Water	145.16 kg/m^3
Fine Aggregate	620.00 kg/m^3
Coarse Aggregate	1130.00 kg/m^3
Water-Cement ratio	0.455
Sporosarcina globiospora (liquid culture)	49.99 kg/m^3

4) 70 mL bacterial sample

Liquid culture used in mix (kg/m^3):

$\frac{69.98}{1.01} = 69.29$

Table 4- Mix design for 70 mL bacterial sample [11]

Cement	430.00 kg/m^3
Water	125.67 kg/m^3
Fine Aggregate	620.00 kg/m^3
Coarse Aggregate	1130.00 kg/m^3

Mixing

Control samples were the standard reference cubes made up of aggregates, cement and water. Twelve control molds were cast having dimensions of 100 mm X100 mm X100 mm. The samples were hand mixed and compacted by tamping. Twenty four cubes of *Bacillus subtilis* and *Sporosarcina globiospora* were cast out of which 4 cubes were for 30, 50 and 70 ml concentrations of the same bacterium. A separate set of 12 cubes were cast using *Sporosarcina globiospora* with ConCure as shown in Fig. (1and 2). Different volumes of bacteria were used in the mix to find the best compressive strength and crack healing effect.

Figure 1- Slump value of approximately 100 mm

Figure 2- Concrete cube blocks in mold [12]

Testing

1) Test for bacterial survival

Three curing compounds were tested for bacterial survival.

a) Super plasticizer MasterRheobuild 1125 (chemical admixture).
b) ConCure (curing compound).
c) Combination of (cement+ sand+ water) as control.

Autoclaved nutrient agar (culture media) is taken in the petri plate. It takes 10-15 minutes for the hot agar to solidify in the plate. After solidification, a bacterial lawn culture is prepared on the petri plate. Then a sterile filter disc impregnated with each of the external compounds is placed on the plate. The plate was incubated overnight at room temperature. Appearance of a zone of inhibition around the disc indicates bacterial rejection of the foreign

compound. Bacterial growth inside the disc indicates that the external compounds do not affect bacterial growth.

2) Compression testing

A fixed amount of cubes were used for compression testing after a period of 7, 14 and 28 days of curing. External load (without shock and impact) is applied on the surface of the cube (other than the top and bottom part). This load is applied on the cube until breakpoint. Unit compressive strength is calculated by – load/sectional area of specimen. Average of three values is taken.

3) pH test

High pH or alkalinity in water contributes to its hardness which leads to scaling or calcium buildup on walls of concrete structures. Increase in pH over a constant period of time causes the water to feel slimy and itchy. Therefore, it is important to periodically monitor pH of cured water from the concrete mix. The pH meter was calibrated with buffer solutions of pH 4 and 7. Then, pH electrode was dipped in the cured water and the display shows the corresponding pH which is documented [8].

4) Presence of ammoniac nitrogen

Ammonia is released as a byproduct of calcite activity. This is a highly corrosive and toxic gas which might come in human contact in the laboratory. Therefore as a precautionary step, periodic tests were conducted to check for the level of ammonia production.

First the distillation apparatus was made ammonia free by distilling ammonia free water. This was done by running 250 mL of sample water followed by 10 mL of phosphate buffer solution. 150 mL of distillate was collected in a flask containing 25 mL of boric acid solution. The final result was 250 mL of ammonia free water.

A standard 0.01N ammonium chloride solution was prepared. 1-15 mL of standard ammonium chloride solution was pipetted into 100 mL Nessler's tubes. 2 mL of Nessler's reagent (potassium tetraiodomercurate (II)) is added to each flask and made up to the mark with ammonia free distilled water. 50 mL of distillate is taken in another Nessler's tube and diluted up to the mark with distilled water after adding 2mL of Nessler's reagent. The obtained color was then matched with standard colors for confirmation.

Ammoniac nitrogen in a sample is given as

$$N = \frac{WX1000}{V} \quad mg/L$$

Where, W=weight of nitrogen in the standard flask matched by sample.

V=volume of sample taken in 100 mL Nessler's tube [7].

5) Effervescence test for calcium carbonate determination

5-10 g of calcium carbonate deposits were scrapped off the concrete control and sample cubes in a test tube. Few drops of dilute hydrochloric acid were added to it. This tube was connected to another tube containing sodium hydroxide. Formation of effervescence indicated the formation of sodium bicarbonate [7].

$$CaCO_3 + 2HCl \rightarrow CaCl + H_2O + CO_2$$

$$CO_2 + NaOH \rightarrow Na_2CO_3 + H_2O$$

6) Flame test for calcium carbonate determination

A small amount of calcium carbonate was taken from the control and the sample concrete cubes in two separate glass discs. Drops of concentrated hydrochloric acid were added to the samples. A glass rod was used to collect this semi-liquid sample and this was exposed to a Bunsen flame. The appearance of a brick red flame confirmed the presence of calcium carbonate [7].

7) Ammonium oxalate test for calcium carbonate determination

Calcium carbonate samples were taken from the control and standard cubes and few drops of dilute hydrochloric acid were added to it. The liquid was collected from the residue and few drops of ammonium oxalate; ammonium hydroxide was added to it. The appearance of white colored precipitate due to the formation of calcium-nitrogen complex confirmed the presence of calcium carbonate [7].

Results and Discussion

1) Effect of curing compounds on bacterial survival

Figure 1- Effect of curing compounds on growth of *Bacillus subtilis*, where 'Rh'-Rheobuild, C+S+W-cement, sand and water, 'Con'- ConCure

Figure 2- Effect of curing compounds on growth of *Sporosarcina globiospora*

There is no zone formation as seen in Fig. (1 and 2) which, indicated that the curing compounds had no effect on bacterial growth and urease production. This also proved that super plasticizer and ConCure can be used in the cement mix to impart specific characteristics to the concrete structure.

2) Compression Testing

Table 5- Compressive Testing results

Sample	Compressive Strength (N/mm^2)		
	7 days	14 days	28 days
Control sample	21.91	28.84	34.43
Sample with 30 mL of *Bacillus subtilis*	17.53	24.68	26.55
Sample with 50 mL of *Bacillus subtilis*	26.16	31.20	32.77
Sample with 70 mL of *Bacillus subtilis*	28.35	34.66	39.83
Sample with 30 mL of *Sporosarcina globiospora*	20.28	26.51	30.79
Sample with 50 mL of *Sporosarcina globiospora*	25.23	29.95	31.85
Sample with 70 mL of *Sporosarcina globiospora*	24.33	33.16	33.60
Sample with 50 mL of *Sporosarcina globiospora* cured using ConCure	24.35	27.15	34.37

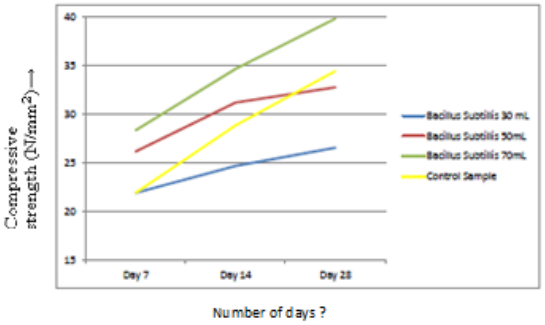


Figure 3- Effect of various concentrations of *Bacillus subtilis* sample on compressive strength of concrete

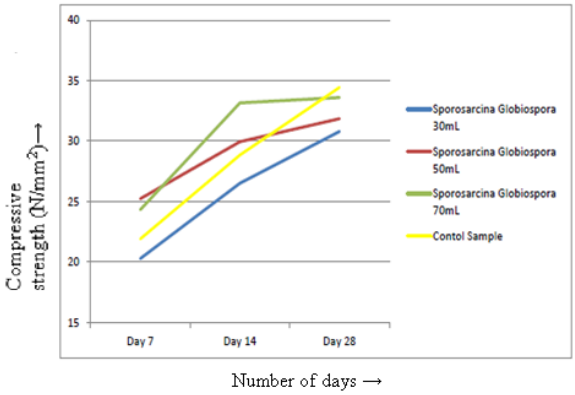


Figure 4- Effect of various concentrations of Sporosarcina globiospora on compressive strength of concrete

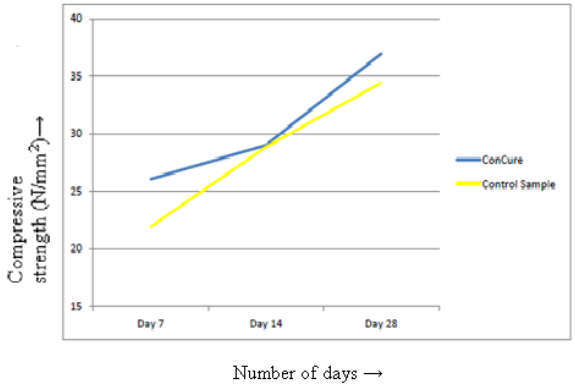


Figure 5- Comparison of compressive strength of cubes with and without ConCure

70 mL concentration of bacterial samples significantly improved compressive strength of the concrete as seen in Table 5, Fig. (3 and 4).

Addition of ConCure to the cement mix improved their mechanical and tensile properties as seen in Fig. (5).

3) Visual confirmation of crack healing

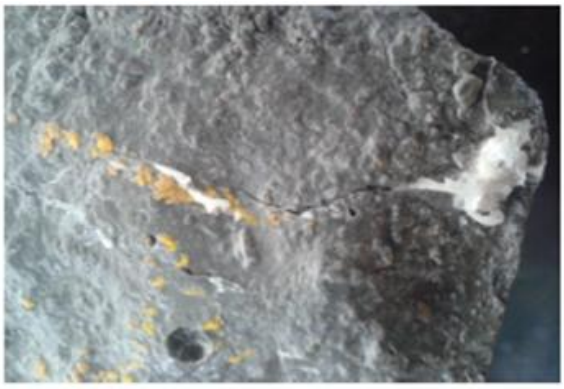


Figure 6- Crack healing in *Bacillus subtilis* after 28 days of compressive testing

Figure 7- Crack healing in *Sporosarcina globiospora* after 28 days of compressive testing

Fig. (6 and 7) confirmed bacterial efficacy in healing concrete cracks.

4) pH testing

Table 6- pH testing of cured water

Sample	pH		
	7 days	14 days	28 days
Control sample	12.34	12.51	12.56
Bacillus subtilis	10.98	10.63	10.52
Sporosarcina globiospora	11.82	11.14	10.83

Table (6) showed that the ureolytic activity of *Sporosarcina globiospora* was slower as compared to *Bacillus subtilis*.

5) Test for presence of Ammoniac nitrogen

Table 7- Ammoniac nitrogen Test results

Sample	Ammoniac nitrogen (mg/L)		
	7 days	14 days	28 days
Control sample	-	-	-
Bacillus subtilis	-	0.8	1.2
Sporosarcina globiospora	-	-	0.4

Table (7) confirmed slow ureolytic activity of *Sporosarcina globiospora*.

Figure 8- Comparison of color of test sample with standard ammoniac nitrogen solution

6) Effervescence test for calcium carbonate determination

(a) (b)

Figure 9- (a) Cured water sample with drops of concentrated hydrochloric acid, b) Effervescence due to change in color of sodium hydroxide.

Effervescence was seen in Fig. 9(b) which is due to change in color of sodium hydroxide. This proves calcite precipitation.

7) Flame test for calcium carbonate determination

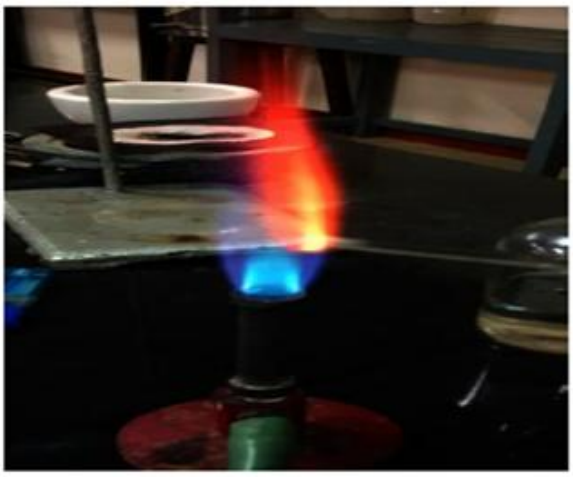
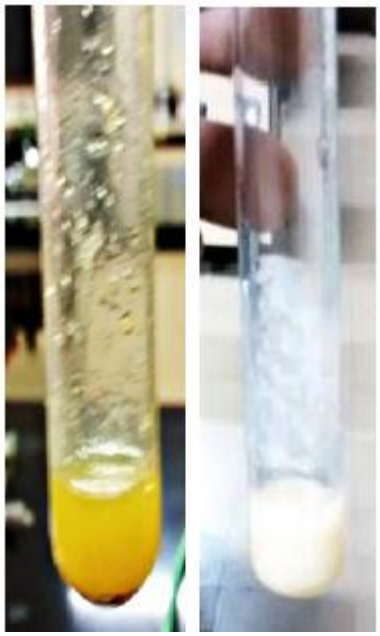


Figure 10- Flame test of cured water for calcium carbonate determination

The appearance of a brick red flame as seen in Fig. (10) confirmed the presence of calcium carbonate.

8) Ammonium oxalate test for calcium carbonate determination



(a) (b)

Figure 11- (a) Cured water sample with drops of concentrated hydrochloric acid, b) Formation of white colored precipitate due to calcium-nitrogen complex.

Formation of a white colored precipitate as seen in Fig. (11-b) due to calcium-nitrogen complex indicates presence of calcium carbonate.

CONCLUSIONS

It was found, that 70 mL concentration of bacterial samples significantly improved compressive strength of the concrete. The results also prove that increasing the curing period of concrete cubes have a positive impact on their strength.

The addition of ConCure (curing compound) to the bacterial concrete mix improved mechanical strength of the concrete. The slight decrease in pH of cured water from bacterial samples confirms carbonic ion production due to urease activity. In case of *S.globiospora*, the decrease in pH over the curing period was slower confirming delayed urease activity. Cured samples of *B.subtilis* produced greater ammoniac nitrogen as compared to *S.globiospora*. Flame test and ammonium oxalate tests confirm calcium carbonate production. All the tests confirm greater urease activity of *B. subtilis* than *S.globiospora*.

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